

## Original Article

# Long-term evaluation of motor function and learning-memory ability in a 3-day-old ischemic brain injury rat model

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Received March 30, 2016; Accepted September 7, 2016; Epub November 15, 2016; Published November 30, 2016

**Abstract:** To evaluate the histopathological changes, physical development and long term motor and learning-memory ability of a 3-day-old rat brain injury model. Postnatal day-3 male Sprague-Dawley rats were randomly assigned to experimental group induced by ligation of bilateral carotid arteries, and sham-operated control group (48 rats/group). At 1 day after the procedure and 3 weeks old, we used hematoxylin-eosin stain, myelin related protein immunohistochemistry, pre-oligodendrocyte marker O4 and TUNEL double immunofluorescence stain to investigate the pathological changes. Inspections of physical development were made after operation until 3 weeks old. Motor activity and coordination of the rats were evaluated by four sensorimotor tests between 3 to 6 weeks old. Learning and memory ability were tested by Morris water maze between 7 to 13 weeks old. The survival rate was 100% (48/48) in the control group and 68.8% (33/48) in the experimental group 24 hours after operation. Pathological changes including periventricular leukoariosis, dilated ventricles, and pre oligodendrocyte apoptosis. The expression of myelin basic protein was reduced at 3 weeks old. Rats in the experimental group showed decreased weight gain ( $P<0.05$ ), poor motor ability and decreased maintenance of balance ( $P<0.05$ ) and learning-memory defects compared with rats in the control group ( $P<0.05$ ). Our rat model revealed a preferential occurrence of brain damage which is similar to preterm infant, and confirmed the early outcome is delayed growth development during childhood. The long term outcome including motor and learning-memory ability defect will persist until adulthood.

**Keywords:** White matter injury, preterm birth, oligodendrocyte, neurobehavioral manifestations

## Introduction

Preterm brain injury has become a significant cause of neonatal brain injury and often leads to severe neurological sequelae that mostly involve motor and visual abnormalities [1], cerebral palsy accounts for up to 20% [2-4]. Preterm brain injury can also widely affect life quality through speech and language problems [5] behavioral, social, attention [6], motor skill [7] and cognitive deficits [8].

Recent studies suggest that approximately 1% of live-born infants are very low birth weight (VLBW), among which the incidence of white matter damage is 20% [9-11]. Over the past 10-15 years, cystic PVL has declined in incidence and currently occurs in less than 5% of VLBW infants [12]. However, the increasing application of MRI to the clinical assessment of

brain injury in the preterm newborn has now revealed that diffuse noncystic white matter injury is the dominant pattern of white matter injury [13, 14], accounting for more than 90% of PVL and occurring in up to half of premature VLBW newborns [12, 15, 16]. Diffuse noncystic white matter injury (WMI) is increasingly being recognized as a risk factor for autism-spectrum disorders, ADHD, and other psychological disturbances [17]. Thus, proper selection of an animal model for studying the pathogenesis involved in preterm brain injury allowing observation of neuromotor and cognitive development is important for studying the mechanism of neuroprotection from brain injury.

A number of animal models have been used for studying premature brain injury [18]. Rodent models based on ischemia-hypoxia are useful because they can mimic the common mecha-

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nism of brain injury in preterm infants that of inappropriate vasoconstriction resulting from the immature vascular system [19]. The most common rodent models are based upon the Vannucci method [20, 21], generally established at postnatal day 7 and 10 with adaptations for younger rats at postnatal day 3 to 6 [22] and postnatal day 6 to 7 mice [23, 24] have also been developed. Carotid artery ligation followed by exposure to low oxygen has recently been successfully applied to study the mechanism of hypoxic-ischemic injury related to immature oligodendrocytes. Lin et al. [25] established a hypoxic-ischemic model in postnatal day 4 rats by bilateral carotid artery ligation followed by 8% O<sub>2</sub> inhalation for 20 min and performed immunohistochemical analysis. The results suggested that pre-oligodendrocytes positively labelled with O4<sup>+</sup> and O1<sup>+</sup> and staining for myelin basic protein decreased and amyloid precursor protein (APP) immunoreaction increased in postnatal day 6 and postnatal day 9 experimental group rats compared to the control group, confirming the occurrence of white matter damage to rats in the experimental group. A previous report [26] by Uehara demonstrated that the decrease of cerebral blood flow was up to about 25% of baseline in the subcortical white matter in neonatal rats with bilateral carotid artery occlusion, which is higher than that in rats subjected to unilateral common carotid artery ligation combined with systemic hypoxia (15%-17%) and which preferentially caused white matter injury and reduced gray matter injury. Back [27, 28] described that cerebral white matter damage is most common at 24-32 weeks gestation in humans, a time at which pre-oligodendrocytes are the predominating oligodendroglial cell [29], whereas in rats it occurs much more frequently on postnatal days 2-4. Thus, it is proper to use postnatal day 2 to day 4 immature rats for study of cerebral white matter injury in preterm infants. Sizonenko [30] has shown that by right carotid ligation followed by 6% hypoxia for 30 min in postnatal d3 rat compromised brain cortical growth and led to a selective alteration of cortical myelinated axons with persistent gliosis. These alterations share neuropathological similarities with the diffuse white matter lesions found in VLBW infants.

So far several studies have evaluated a brain injury model established by bilateral carotid artery ligation in 3-day-old newborn rats [30,

31], but no one has follow up longer enough to evaluate the long-term motor and cognitive development which may provide important information for preterm brain injury. In this study, we established the hypoxic-ischemic brain injury model by ligation of bilateral carotid arteries in postnatal day 3 rats with the aim of investigating the histopathological changes, short-term physical development and long-term motor and learning-memory ability.

### Materials and methods

#### *Animals and experimental groups*

96 clean male Sprague-Dawley (SD) rats aged 3 days were obtained from the Laboratory Animal Center of Shanghai Medical College, Fudan University, weighing 7.5-12 g, which were divided into 2 groups: sham operation group (normal control) and bilateral carotid artery ligation group (experimental group). This experiment was undertaken with approval from Fudan University Affiliated Children Hospital animal use and care committee.

#### *Model establishment*

The rats were anesthetized with diethyl ether. In a supine position, the skin of the neck was sterilized with disinfectant, and a 0.5 cm incision was made at the cervical ventral midline with an aseptic scalpel under sterile conditions. The subcutaneous tissue was separated layer by layer until the euro vascular bundle was found and bilateral common carotid arteries were separated. The skin was then sutured for the control group and the bilateral carotid arteries were ligated with 9-0 sutures for the experimental group followed by skin suturing. The rats were allowed to recover to their normal body temperature and activity using a water bath at 37°C after the operation before being placed back to the cage. 31.2% (15/48) of rats in the experimental group died during or right after surgery resulting from intolerance to the surgical treatments.

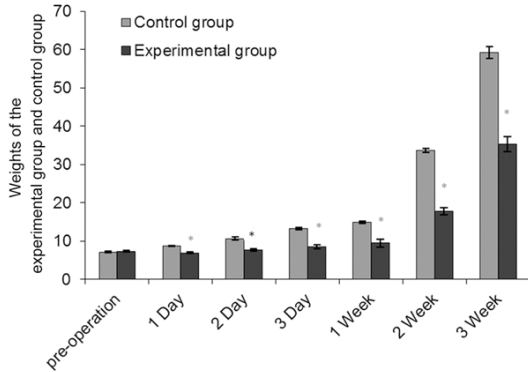
#### *Evaluation of growth and development*

We randomly selected 8 live rats from each group after operation until 3 weeks old and recorded their weight, eye opening, ear erecting, and incisor eruption by daily video footage.

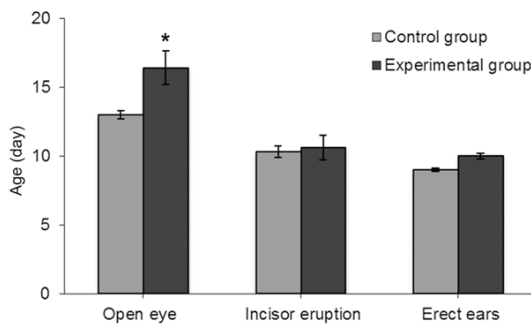
#### *Motor development assessment*

We randomly selected 8 live rats from each group at 3, 4, 5, and 6 weeks of age. Four sen-

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**Figure 1.** Weights of the experimental group and control group. A significant difference in weight between the two groups was seen every day until 3 weeks after the procedure (\* $P < 0.05$ ).



**Figure 2.** Physical development of experimental group and control group. Eye lid opening for rats in the experimental group happened obviously later than that for rats in the control group. The difference was statistically significant (\* $P < 0.05$ ). Other physiological phenomena such as incisor eruption and erect ears were not statistically significant.

sorimotor tests including the vertical screen test, foot fault test, beam traversal test and rotarod test were performed twice per week and the whole process for each test was recorded by video. A neurologist from the Children's Hospital of Fudan University evaluated the video footage. Selected reflexes and evaluation criteria were based on Wang [32]. Results were taken as means of the two experiments in a week. The apparatus and software were from Jing Mei Company, Shanghai.

### Learning and memory assessment

We randomly selected 8 live rats from each group at 7, 9, 11 and 13 weeks of age to assess the learning and memory ability by water maze. During training period, 8 live rats from each

group were allowed to swim in the water maze three times a day and two minutes for each time from each quadrant. If some of the rats can't find the platform in the water within two minutes, then help them to. After finishing the training period, the Morris water maze test [33] was carried out once in two weeks to determine the cognition and memory of rats. The water was stained by ink so that the platform can't be seen above the water. The tracking software automatically recorded original data and analyzed swim speed, swimming time around the platform, swimming curve and success rate on the platform. The water maze and software were from Jing Mei Company, Shanghai.

### Histology

At 24 hours after the procedure and at postnatal 3 weeks, we selected 8 live rats from each group for histological study. After being anesthetized by ether, the rats were perfused with normal saline and 4% PFA successively, by left ventricular catheterization. Next, we carefully stripped the brain tissue and subsequently fixed the tissue in 4% PFA for 48 hours. Brain tissue was dehydrated by ethanol and then paraffin-embedded. Continuous coronal sections of 6- $\mu\text{m}$  thickness were taken and analyzed in the anterior commissure lateral ventricle plane. Changes in the structure of brain tissue were observed after hematoxylin-eosin staining of the brain tissue section including the histological profile of the cortex, subcortex, periventricular white matter, and ventricles under a light microscope.

Myelin basic protein (MBP) immunohistochemical staining was conducted to compare the difference in staining of the subcortical white matter versus the periventricular white matter. MBP antibody was from ABCAM Company, British. We used the TUNEL assay to compare the number of apoptotic cells in the subcortical and periventricular areas of the two groups under a fluorescence microscope. The nuclei of apoptotic cells emitted yellow-green fluorescence under a fluorescence microscope (TRITIC with an emission wavelength of 620 nm and an absorption wavelength of 550 nm). Pre-OL marker O4 immunohistochemical staining was conducted to compare the number of pre-OL cells in the subcortical and periventricular area of the two groups under the fluorescence microscope. TRITIC emission was used to detect pre-

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**Table 1.** Tests for 3-day-old neonatal rats after bilateral carotid artery ligation at different ages

Test	Group (n=8)	3 weeks	4 weeks	5 weeks	6 weeks
Vertical screen test (s)	Control	16.6±0.2	28.0±0	29.4±0.4	30.0±0
	Experimental	4.6±0.8**	4.5±0.6**	5.2±0.5**	5.3±0.7**
	<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
Total number of steps in foot fault test (step)	Control	68±0.2	73±0.4	90±0.1	95±0.2
	Experimental	59±0.2*	72±0.2	91±0.1	88±0.1
	<i>P</i> value	0.0346	0.4571	0.5332	0.0551
Forelimb false step rate for in foot fault test (%)	Control	2.5±0.1	1.1±0.2	0.45±0.2	0.2±0.1
	Experimental	3.8±0.2**	2.2±0.2**	0.75±0.1*	0.7±0.2**
	<i>P</i> value	0.0087	0.0075	0.0343	0.0024
Hind limb false step rate for in foot fault test (%)	Control	2.3±0.2	0.8±0.1	0.3±0.1	0.1±0.1
	Experimental	2.2±0.1	1.2±0.2*	0.4±0.1*	0.3±0.1*
	<i>P</i> value	0.5714	0.0238	0.0447	0.0171
Score on beam walking test	Control	0.2±0.1	0.1±0.1	0.1±0.1	0.1±0.1
	Experimental	1.8±0.2**	1.4±0.2**	1.3±0.2**	1.3±0.1**
	<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001

\**P*<0.05 vs control, \*\**P*<0.01 vs control.

**Table 2.** Rotarod test for 3-day-old neonatal rats after bilateral carotid artery ligation at different ages

	Group (n=8)	4 weeks	5 weeks	6 weeks
Rotarod distance (cm)	Control	682.9±0.3	850.6±0.2	1027.3±0.4
	Experimental	285.8±0.7*	339.8±0.6*	559.5±0.7*
	<i>P</i> value	0.0353	0.0327	0.0191
Rotarod speed (cm/s)	Control	8.3±0.6	11.6±0.5	13.0±0.6
	Experimental	6.0±0.7*	8.4±0.3*	9.3±0.1*
	<i>P</i> value	0.0314	0.0256	0.0212

\**P*<0.05 vs control.

## Results

The survival rates of the control group and experimental group 24 hours after operation were 100% (48/48) and 68.8% (33/48), respectively. Between 1 and 2 days after operation all the experimental group rats showed cyanosis, decreased physical activity and reduced diet.

3% (1/33) of rats in the experimental group suffered from convulsions after the operation and died 48 hours after operation. Among the experimental group, within one week after operation, 28.1% (9/32) of rats suffered from monoplegia and circled to the healthy limb side while crawling, and 12.5% (4/32) of rats had paralysis of the lower limbs, leading to falling down when crawling.

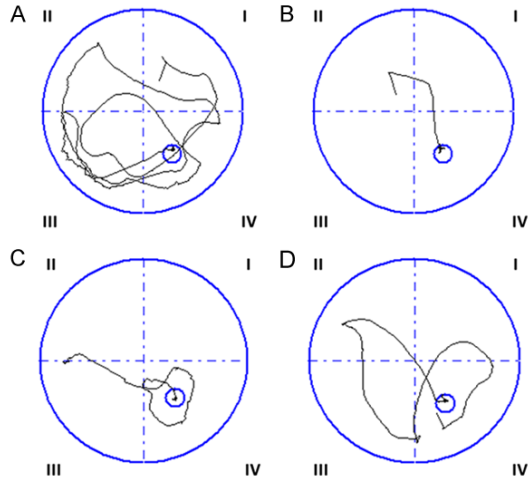
Before the procedure, there was no significant difference in body weight of rats between the control group and the experimental group. At the first day after the procedure, all rats in the experimental group experienced a decrease in body weight. The average body weight before the procedure was 7.11±0.16 g in the experimental group and 7.28±0.25 g in the control group, and the average body weight after the procedure was 6.92±0.16 g and 8.71±0.17 g,

OL cells, and a laser confocal scanning microscope to observe O4 and TUNEL double positive cells. The O4 antibody was from R&D systems Company. Fluorescence microscope was Leica Fluorescence Microscope, USA. The laser confocal scanning microscope was Leica TCS SP2, USA.

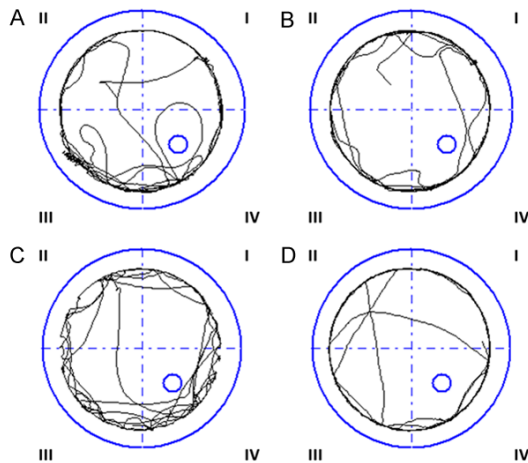
## Statistics

Normally distributed continuous variables are presented as mean ± standard deviation (mean ± SD) and were compared using the independent sample *t*-test. The differences were considered statistically significant when the *P* value was less than 0.05. Statistical analysis was performed by SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Water maze tracking software was used to analyze learning and memory in rats.

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**Figure 3.** The swimming route of control rats in the Morris water maze. A. Rats go into the water at the first quadrant. B. Rats go into the water at the second quadrant. C. Rats go into the water at the third quadrant. D. Rats go into the water at the fourth quadrant.



**Figure 4.** The swimming route of experimental rats in the Morris water maze. A. Rats go into the water at the first quadrant. B. Rats go into the water at the second quadrant. C. Rats go into the water at the third quadrant. D. Rats go into the water at the fourth quadrant.

respectively. Subsequently, the rats in the experimental group slowly gained weight, with an average weight of  $7.55 \pm 0.24$  g at day 2 after the procedure compared with  $10.55 \pm 0.23$  g in the control group. A significant difference in weight between the two groups was seen every day until 3 weeks after the procedure ( $P < 0.05$ ) (**Figure 1**). Eye lid opening for rats in the experimental group happened obviously later than that for rats in the control group at  $16.4 \pm 1.2$

and  $13.0 \pm 0.3$  days, respectively (**Figure 2**). The difference was statistically significant ( $P < 0.05$ ). Other physiological phenomena such as incisor eruption which appeared at  $10.3 \pm 0.4$  days for the control group and  $10.6 \pm 0.9$  days for the experimental group, and ear erecting that appeared at  $9.0 \pm 0.1$  days for the control group and  $10.0 \pm 0.2$  days for the experimental group were not statistically significant (**Figure 2**).

### Vertical screen test

The vertical screen test was performed twice a week in rats 3 weeks after birth to assess forelimb and hind limb strength. Results for a week were taken as means of the two experiments within that week. Time of seizing the screen for the control group gradually became longer as their age increased. The ability to grasp for the experimental group developed slowly and time of seizing the screen was obviously decreased compared to the control group. The difference between the two groups was statistically significant ( $P < 0.01$ ) (**Table 1**).

### Foot fault test

The foot fault test was used twice a week in rats 3 weeks after birth to evaluate their motor coordination. Results for one week were shown as means of the two experiments within that week. Compared with the control group, the total number of steps for the rats in the experimental group was less at 3 weeks' postnatal age ( $P < 0.05$ ), (**Table 1**). In contrast, no statistically significant differences were observed when testing at 4, 5 and 6 weeks' postnatal age. Rats in the experimental group made more false steps than that in the control group. False steps rates for forelimb and hind limb were calculated. Higher false steps rates were seen in the experimental group compared to the control group and the difference was statistically significant ( $P < 0.05$ ) (**Table 1**).

### Beam traversal test

The balancing ability of rats in the experimental group obviously decreased compared to the control group. The beam traversal test was used for rats 3, 4, 5 and 6 weeks old. The score in the experimental group was much higher than in the control group and the difference was statistically significant ( $P < 0.05$ ) (**Table 1**). Most of the rats in experimental group grasped



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**Table 3.** The percentage of time around the platform for 3-day-old neonatal rats after bilateral carotid artery ligation at different ages in water maze test

Test	Group (n=8)	7 weeks	9 weeks	11 weeks	13 weeks
Percentage of time around the platform (s)	Control	62%±0.7%	63%±0.4%	61%±0.6%	72%±0.3%
	Experimental	7%±0.7%**	19%±0.6%**	19%±0.8%**	12%±0.2%**
	<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001

\*\*P<0.01 vs control.

**Table 4.** Swim speed of 3-day-old neonatal rats after bilateral carotid artery ligation at different ages in water maze test

Test	Group (n=8)	7 weeks	9 weeks	11 weeks	13 weeks
Swim Speed (mm/s)	Control	168.4±0.3	153.3±0.2	152.1±0.8	121.4.0±0.3
	Experimental	139.3±0.9*	123.3±0.6*	78.0±0.1**	29.7±0.6**
	<i>P</i> value	0.0336	0.0314	0.0078	0.0046

\*P<0.05 vs control, \*\*P<0.01 vs control.

one side of the beam or rotated more than 90 degrees when traversing the beam. 12.5% of the experimental rats (1/8) fell down from the beam. In contrast, rats in control group revealed normal motor action and 100% (8/8) of them could reach the platform located at the end of the beam.

### Rotarod test

The rotarod distance and rotarod speed for control rats increased with age. Rats in the experimental group showed shorter rotarod distance and lower rotarod speed in comparison of control group (P<0.05). In the experimental group, the ability to remain on the rotating rod increased slowly and shorter rotarod distance and lower rotarod speed were also required in comparison with the control group (Table 2) (P<0.05).

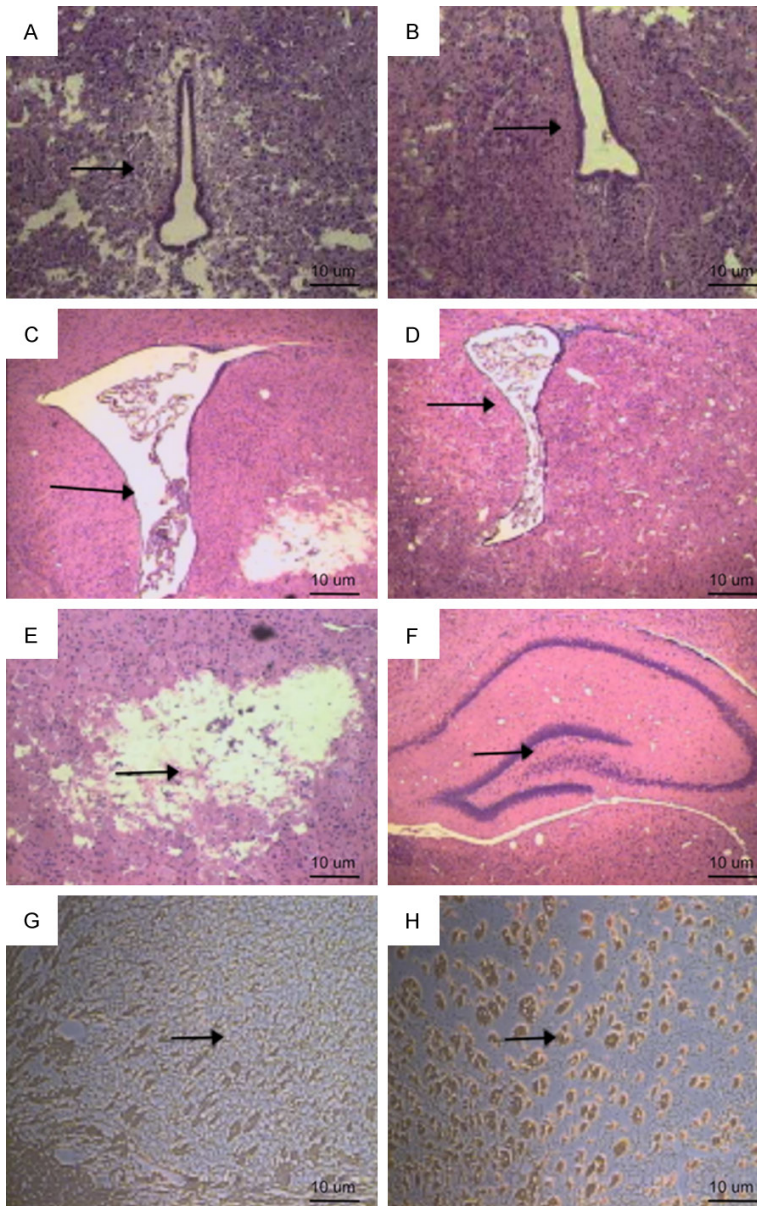
### Water maze test

Rats without memory of the platform will swim along the pool in a circular motion while rats with spatial cognition and memory of the platform change their swimming pattern and look for the location of the platform (Figures 3 and 4). The success rate of getting to the platform was 93.8% (8/8) for control group rats and 25% (2/8) for experimental group rats, suggesting a lower cognition ability of rats with brain damage. The longer time the rats spend in the quadrant of the platform and the higher the percentage of time around the platform, the stronger the cognition function of the rats.

The experimental group spent shorter time on the platform compared to control group and the difference was statistically significant (P<0.05) (Table 3). The parameter of movement velocity was used to assess motor function. Movement velocity of the experimental group was slower than that of the control group and the difference was statistically significant (P<0.05) (Table 4).

### Comparison of histology

HE staining showed that brain lesions altered with age. 24 hours after operation, subcortical and periventricular leukoariosis occurred in the experimental group. Damage to the cortex and ventricular dilatation was not obviously observed in the experimental group compared to control group. Ventricular dilatation and leukomalacia appeared in rats in the experimental group at 3 weeks postnatal age. All experimental group rats suffered from gliocyte hyperplasia to varying degrees (Figure 5A-F). MBP immunohistochemical staining results did not show any difference between control and experimental groups 24 hours after operation due to the immature myelination. As the myelination matures with age, the difference between control and experimental groups became more and more evident. Gray matter myelin was evidently seen in the corpus callosum and internal capsule of the rats from the experimental group, but not the control group rats which showed a sparse pattern of gray matter myelin (Figure 5G, 5H).



**Figure 5.** Pathology in rat brain tissue. A. Periventricular leukoaraiosis is seen in the experimental group at 24 h after surgery. B. No periventricular abnormalities in the control group at 24 h after surgery. C. Dilated brain ventricles in the experimental group. D. Brain ventricles are normal in the control group. E. Damaged intracranial structure. F. Intact intracranial structure in the control group. G. Positive staining of myelin basic protein (MBP) was occasionally seen in the control group. H. Positive staining of MBP was obviously seen in the control group.

The experimental group showed higher numbers of apoptotic cells than the control group 24 hours after operation. Oligodendrocytes labeled with anti-O4 were detected by TRITC excitation. Observation of O4 and TUNEL-double-positive cells were processed using confocal laser microscopy. Our results suggest-

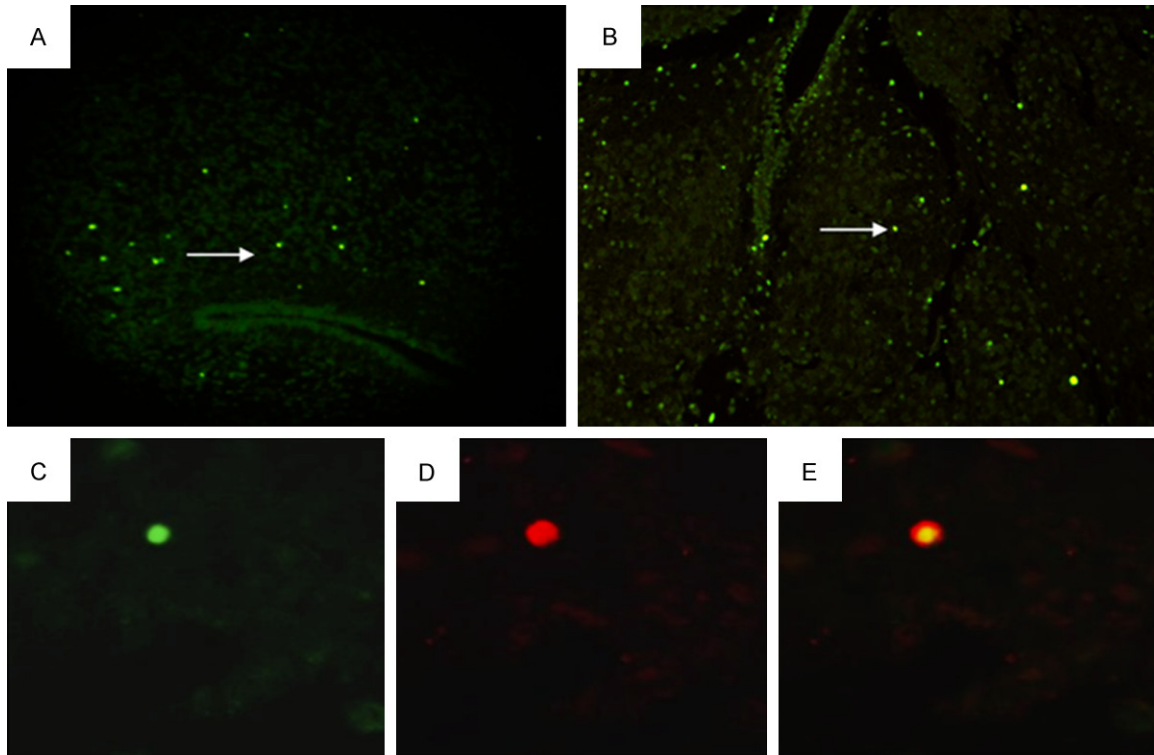
ed that the apoptotic cells were mainly oligodendrocytes (Figure 6).

### Discussion

The aim of this study was to evaluate the histopathological changes, short-term physical development and long-term motor and learning-memory ability of a 3-day-old rat ischemic brain injury model. We revealed declined growth and development, motor function and learning-memory abilities, increased white matter disruption, enhanced oligodendrocyte apoptosis and scattered distribution of myelin.

Methods of assessing neurobehavioral development for rats have been well established and successfully applied for the study of neurobehavioral sequelae of traumatic brain injury. Recently they have also been used to study neurobehavioral outcomes after hypoxia-ischemic brain injury. Back *et al.* [34] established the hypoxia-ischemic brain injury model by unilateral common carotid artery ligation followed by exposure to systemic hypoxia with 8% oxygen for 2 h in 7-day-old rats, in which body weight, primitive reflexes as well as motor coordination development between experimental and control group rats were compared. And they concluded that neonatal rats with hypoxia ischemic brain injury suffered from retarded growth and development, delayed eyelid

opening, primitive reflexes inhibition, limb movement disorder and declined motor coordination. Our results also showed declined growth and development of rats in the experimental group. Weight loss is associated with water deprivation caused by reduced food intake and consciousness depression after



**Figure 6.** Apoptosis of oligodendrocytes at 24 hours after surgery (arrows indicate apoptotic cells). A. Fewer apoptotic oligodendrocytes in the control group. B. More apoptotic oligodendrocytes in the experimental group. C. Apoptotic cells (green). D. O4 positive cells (red). E. O4 and TUNEL double positive cells (yellow).

brain injury, and may also relate to a decreased appetite from ischemic damage to feeding center in hippocampus. In the current study we found one rat in the experimental group suffered from convulsions. Because all of the experimental group rats must be returned to their cages for maternal feeding, which was unfavorable for observation of convulsions, it is difficult to calculate the incidence of convulsions. Eyelid opening and eyelid reflex were obviously delayed in rats in the experimental group compared to the control group. Whereas differences in time spent on pricking ear and auricle reflex were not statistically significant.

Poggi *et al.* [35] developed a white matter damage model by intracranial injection of lipopolysaccharide (LPS) in rats and evaluated behavioral and motor development. Rats were intracervically injected with LPS or saline and evaluation for motor developmental milestones was performed in neonatal rats on days 1 to 21 after delivery. On postnatal day 21, animals were sacrificed for immunohistochemistry detection. The results suggested that there was no significant difference in neonatal weight, bal-

ance function, negative geotaxis, forelimb grasp, audio startle, eye opening and activity. Surprisingly, neonatal rats subjected to LPS injection revealed better ability of forelimb placement and balance. Although the immunohistochemical findings revealed cerebral white matter damage, there was no obvious difference in the motor outcome of neonatal animals, relating to manifestations of cerebral palsy in humans. This could be due to the strong compensatory adjustment of neonatal rats after brain injury induced by injection of LPS.

In our study, rats with hypoxia ischemic brain injury also showed a recovery of motor function at certain time points. There was no significant difference between the experimental group and control group in the age at which ear unfolding, and incisor eruption occurred, suggesting a better compensatory adjustment in rats than in humans. Therefore, some scholars believe that the rat model is not suitable for study of brain injury. However, some other researchers have applied certain motor tests [36, 37] (such as wheel-running test and stair climbing test) in the neonatal rats after hypoxic-ischemic



brain injury with long-term follow-up, and detected motor and cognitive deficit in the experimental group rats.

The Morris water maze test was mainly for study of cognition and memory. We combined the Morris water maze test with motor tests, which could comprehensively evaluate neuro-functional changes of neonatal rats after brain injury. In this study, for the first time we applied brain injury model by bilateral carotid artery ligation in 3-day-old newborn rats and evaluated long-term motor outcomes and cognition development of model animals, which would provide a basis for intervention study on hypoxic-ischemic brain injury in preterm infants using immature rat model. Ikeda *et al.* [38] suggested that neonatal rats with cerebral white matter injury revealed a sustained cognitive impairment during infancy, adolescence and maturity. Mishima *et al.* [39] set up the hypoxia-ischemic brain injury model by unilateral common carotid artery ligation followed by exposure to systemic hypoxia in 7-day-old rats and evaluated the cognition development by 8-arm radial maze task between 2 and 17 weeks after operation. The results suggested that the cognitive level 16 weeks after surgery was more severe than that for 3 weeks in experimental group rats, confirming that cognitive impairment slowly progressed with time. In our study, the Morris water maze test was carried out in rats after birth for 7, 9, 11 and 13 weeks, equivalent to adolescence and maturity in humans, and the results showed decreased swim speed, shorter residence time on the platform, and lower success rate on the platform in rats subjected to bilateral carotid artery ligation compared to sham-operated rats, indicating sustained spatial cognition function and long-term memory defects.

In summary, our present study established a brain injury model by bilateral carotid artery ligation in 3-day-old premature rats and the histological findings revealed a preferential occurrence of cerebral white matter damage. Results for assessment of motor function and cognition revealed retarded growth and development, inhibition of primitive reflexes, decreased physical activity, even convulsion and paralysis in the early stage, and cognition dysfunction in the later stage in the experimental rats. These results suggest that the model demonstrated a similar condition of brain injury as that found in preterm brain injury in human.

### Disclosure of conflict of interest

None.

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